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Anticancerous Activity of Chloroform and Ethyle Acetate Extract of *Solanum nigrum L.* against B16F10 Cell Lines in C57BL/6 mice

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Abstract: **Medicinal plants have been used for decades for the treatment of different diseases. *Solanum nigrum L.* is an important medicinal plant in many traditional health care system. It has been used for various diseases like liver disorder, diabeties, infections, cancer, inflammations and peptic ulcer. In the present study, we screened the whole plant of *Solanum nigrum L.* for their *in-vivo* anticancerous activity.**
Result: After in-vivo screening of the extract, it was concluded that the aqeous chloroform extract of *Solanum nigrum L.* revealed a marked cytotoxicity against B16F10 cell lines with IC_{50} values 346.1 µg/ml (Romana *et al.*,2014). Chloroform extract showed the lowest IC_{50}, 346.1µg/ml as compared to ethyl acetate extracts having IC_{50} values 355.5 µg/ml. IR and GC-MS spectral analysis confirms the presence of solanine in the extract which is an anticancerous alkaloid.

Keywords: **Medicinal plant, *Solanum nigrum L.*, extract, cytotoxicity.**

1. INTRODUCTION

Cancer is a disease in which a cell or a group of cells represents uncontrolled growth invasion. Cancer is one of the most life threatening diseases and serious public health problems in both developed and developing countries (Poonam *et al.*, 2012).

Cancer can be treated by surgery, radiation therapy, chemotherapy and immunotherapy. Cancers that are most often cured are breast, cervix, prostate, oral, colon and skin, if they are diagnosed early. Improving the quality of life of patients living with cancer and dying from cancer is therefore an urgent humanitarian need.

Solanum nigrum L. (Kaambal)(Kashmiri) has been traditionally used to treat pathological ailments like fever, ulcers, bacterial infections, fungal infections, jaundice and liver disorders (Creasy *et al.*,1981; Capizzi *et al.*, 2003; Sudhir *et al.*, 2000 and Borgia *et al.*, 1981).

The history of *Solanum nigrum L.* dates back to ancient China and the Mediterranean region as a highly popular laxative drug and a general tonic (Dashputre *et al.*, 2010). It is used as purgative and astringent tonic; its stimulating effect combined with apparent properties renders it especially useful in tonic dyspepsia (Chintana *et al.*, 2012). Powdered roots are sprinkled over ulcer for healing. Leaf and berries are eaten either raw or boiled, sprinkled with salt and pepper. Some workers have worked out anticancerous activity of *Solanum nigrum* L. (Anindyajati *et al.*, 2010) but very little is known about the mechanisms involved.

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2. MATERIALS AND METHODS

In the present study the whole plant of *Solanum nigrum L.* was collected from the local surrounding at Bhopal district of (M.P) during the months of October-November, 2012. A voucher specimen was submitted in the herbarium at the P.G. Department, Unique College, Bhopal, M.P, India, where it was authenticated by Dr. Jagrati Tripathi, Professor and head department of biotechnology and a herbarium number 280 was assigned to it. The specimen was kept in the herbarium of the said department for future references.

Extraction Procedure:

The plant *Solanum nigrum* L. was collected and washed thoroughly under running tap water and then rinsed in distilled water and allowed to dry for some time. Then the plant was shade dried without any contamination for about 3 to 4 weeks. The powder was extracted according to (Rashmi *et al*., 2010). The dried plant was powdered (coarse) and subjected to Soxhlet appratus using ethyl acetate and chloroform respectively. Almost all the chlorophyll and lipid is deposited on the side of the flask and was removed carefully. The extraction was done with each solvent until the supernatant in the Soxhlet became transparent for 36 hours. Every time before taking the solvents of higher polarity to remove the traces of previous solvents, exhausted marc was completely dried. All the extracts were filtered, dried and weighed.

***In-vivo* Anticancerous activity:**

Experimental Animals:

Healthy C57BL/6 Mice (Both Sex) weighing between 180-250gm were selected for study. Animals were maintained at 25 ± 2^0C and kept in well ventilated animal house under natural photoperiodic condition in polypropylene cages with paddy husk as bedding with free access to food and water. During the period of experiment the animals were fed with standard feeding pellets**.**

The experimental protocol described in present study was approved by Institutional animal ethical committee (IAEC) PBRI Bhopal. The experiment was conducted as per the permission of Institutional animal ethical committee (IAEC) of (**Reg No1283/C/09/CPCSEA).** All conditions were maintained according to CPCSEA norms.

Acute oral toxicity study:

Determination of LD_{50} value:

The initial doses of extracts for the pharmacological study were about 1/10th of the maximum tolerated safe dose found from acute toxicity studies. They were administered once daily by oral route. For the second dose; the initial dose was incremented by twice. The study is needful before pharmacological screening on animals.

The acute oral toxicity study was carried out according OECD (Organization for Economic Co-operation and Development) 423 guideline which is based on a stepwise procedure with the use of a minimum number of animals per step. Absence or presence of compound related mortality of the animal's dose at one step will determine the next step.

Healthy, young, Swiss albino mice of either sex (150-250g) were used for this study. Animals were fasted prior to dosing (only water was withheld over night). On next day, the fasted body weight of each animal is determined and the dose is calculated according to the body weight.

20 animals were divided into four groups for giving dose 05, 50, 300 and 2000 mg/kg. First of all, examined changes in animals after giving dose 100 mg/kg, rodents not died (means dose was tolerated) so subsequent doses were increased by a factor of 0.7. Maximum dose is 2000mg/kg.

Animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hours), and daily thereafter, for a total of 14 days.

Method:

Hair of dorsal side in a particular area was removed by hair remover (1x 1cm) of each mouse. All mice were kept in a laminar airflow cabinet under pathogen free condition throughout the tumor implantation. The implanted tumor from tumor donors (mouse having melanoma tumor) was removed from the mice. Tumor samples are dissected free of necrotic tissue and blood vessels and were cut into small pieces of about $8mm^3$ mechanically and it was munched with the help of bent scissor. Cell suspension was stained with trypan blue stain. All cells which were not stained were counted using haemocytometer. Cell suspension of 5 lack cells/animal was implanted S.C for each mouse at the hair removed area. The mice bearing the implanted tumors were randomly divided into 3 groups with 6 mice in each group.

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Experimental Design:

Mice (n = 18) were randomized into following groups:

Group 1- Kept as Control (Vehicle) treated with (0.2ml DMSO)

Group 2- Treated with 200mg/kg extract of *Solanum nigrum plant* (p.o.).

Group 3- Treated with 50mg/kg isolated component of *solanum nigrum* (p.o.). **Evaluation of tumor Growth:**

During the treatment, the size of the implanted tumors was measured by vernier caliper to construct the tumor growth curve *in vivo*. The control group was kept on normal diet with DMSO only. The test group was given dose of 200mg/kg body weight throughout the experiment daily orally. Tumor volume was calculated by the formula:

$$\textbf{Tumor volume = length X width}^2\textbf{/ 0.5}$$

Tumor volume doubling time is defined as the time required for the tumors to grow from $50mm^3$ to $100mm^3$ for the control group and from $35mm^3$ to $70mm^3$ for the treatment group, respectively. The tumor growth delay time is estimated as the time difference for the treated tumors and the controls to reach a volume of $70mm^3$

Biostatical interpretation:

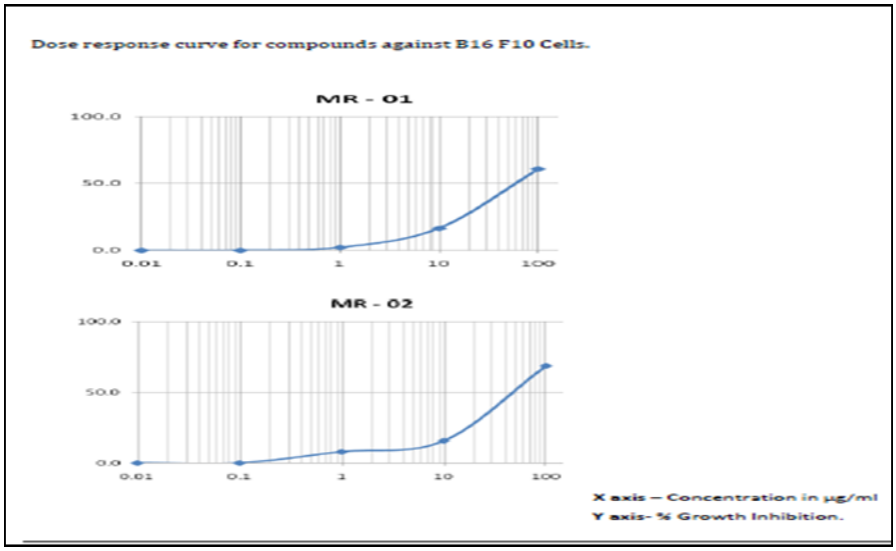
All data were analyzed by One Way ANOVA followed by Bonnferroni test. $P<0.05$ was considered as level of significance.

3. RESULTS AND DISCUSSION

***Invitro* (B16F10) Results:**

In-vitro studies on *Solanum nigrum* L. extract depicted significant anticancerous activity against mice melanoma cancer cell line (B16F10). Percentage of viable cell was obtained by performing trypan blue dye exclusion technique. The cytotoxicity activity was carried out by using MTT assay.

To measure the cytotoxicity of both (ethyl acetate and chloroform) aqueous extract of *Solanum nigrum* L. leaves in human melanoma cell line (B16F10). The cells were cultured with (0.01, 0.1, 1, 10, 100, µg/ml) or without extract for 12hrs to 96hrs. Cell viability was evaluated by trypan blue dye exclusion method. By using trypan blue test, aqueous extract exhibited a remarkable reduction against B16F10 cell viability in a concentration dependent manner with $p<0.05$ Vs control, these results are in concordant with MTT assay. Thus inhibition of cell growth by aqueous extract was more pronounced at concentration of (100ug/ml). Therefore potential growth inhibiting activity of aqueous extract should be considered for further phytochemical analysis and marker compound identification.The IC_{50} value of ethyl acetate and chloroform extract were 60 µg and 50 µg. Hence the chloroform extract shows the best anticancerous activity, so that it could be used for further development as a cancer therapeutic against Human melanoma as alone or in combination with other chemotherapeutic drugs.



Graph : Representing Significant activity of Ethyl acetate and Chloroform extract of *Solanum nigrum* L. against B16F10 cell line

In *in-vitro* anticancer investigation IC_{50} for ethyl acetate extract and chloroform extract was observed to be 60 µg/ml and 50 µg/ml respectively. This revealed that both extract were having significant anticancer potential, but amongst them chloroform extract was more potent and hence it was selected for further *in-vivo* investigation

LD_{50} determination indicates safety profile of the drug. In case of extract no mortality was observed till 2000 mg/kg. Incase of isolated component mortality was found.Hence, 2000 mg/kg was considered as Not Observed Adverse Effect Limit (NOAEL) for present set of experiment. Dose was selected accordingly as safe dose. Results of anticancerous activity of aqueous extract of *Solanum nigrum* L. showed that extract exhibited significant anticancerous activity. All animals (C57BL/6) were having lethargy behaviour during the experiment duration. The animals were given drug per oral (p.o.) for test sample and cancer cells were injected subcutaneously (s.c.) from cell suspensions. DMSO (Dose-0.2ml) was used as vehicle for control group. The P ‹ 0.05 was considered as level of significance. P was found to be less than 0.05 so difference between the groups was considered to be significant.

The results are presented as means ±S.D. Significant differences between control and treatment groups were calculated using One way ANOVA followed by Dunnet's test number of replicates (N) = 6. *P* values of ≤ 0.05 were considered significant.

Table: Showing difference in control, test groups; 200mg/kg and 50mg/kg respectively with standard deviation.

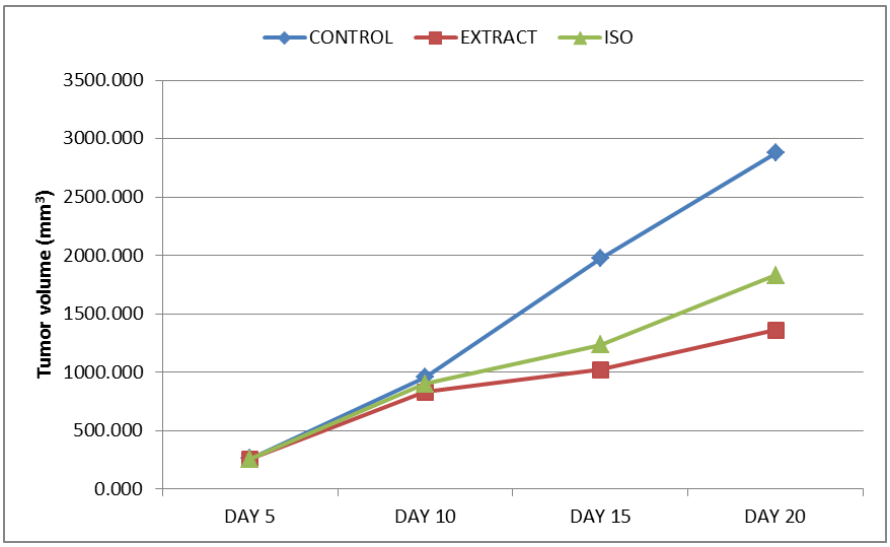
Test group	Tumor volume (mm^3)*			
	5 day	**10 day**	**15 day**	**20 day**
CONTROL (0.1 mg/kg)	264.50±69.67	958.66±88.61	1976.500±84.18	2880.667±177.00
EXTRACT (200mg/kg)	258.83±40.88	832.667±82.70[a]	1022.333±56.22[b]	1363.167±136.95[b]
ISO (50 mg/kg)	257.16±23.77	901.667±16.99	1234.000±73.31[b,c]	1832.500±72.67[b,c]

*All data presented in Mean±SD(N=6)

[a] P<0.05 as compared to control group.

[b]P<0.001 as compared to control group.

[c] P<0.001 as compared to extract group.



Graph: Representing tumor difference in control, testgroups;200mg/kg and 50mg/kg respectively.

In present investigation safety profile of extract was ascertained according to acute oral toxicity. In this concern OECD 423 guideline was adopted. Accordingly four doses were selected 5mg/kg, 50 mg/kg, 300mg/kg and 2000 mg/kg. No mortality was observed till 2000 mg/kg, hence for the selected extract 2000 mg/kg was considered to be No Observed Adverse Effect Limit (NOAEL). $1/10^{th}$ of NOAEL (2000 mg/kg), i.e. 200 mg/kg was selected as dose for further *in vivo* investigation of extract. For isolated component LD50 was found to be 50mg/kg, hence its $1/10^{th}$, i.e. 200 mg/kg was

selected as dose for in vivo investigation. Results of anticancerous activity study of aqueous extract of *Solanum nigrum* L. showed that extract exhibited significant antitumor activity. All animals (C57BL/6) were having lethargy behaviour during the experiment duration. The animals were given drug per oral (p.o.) for test sample and cancer cells were injected subcutaneously (s.c.) from cell suspensions. Tumor volume was measured each week for next 20 days. In animals of control group there was exponential increase in tumor volume after 5^{th} day. In animals treated with extract and isolated component there was significant variation ($p<0.05$) in tumor volume as compared to control group. Tumor volume in isolated component was significantly less ($p<0.05$) as compared to extract treated group. There are two most probable reasons for the said results, firstly there may be presence of more active bioactive component in crude extract as compared isolated component, and secondary presence of synergism can be attributed for the same.

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